



University of Groningen

Acetylcholine release in the hippocampus

Moor, Eitan

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

1998

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Moor, E. (1998). Acetylcholine release in the hippocampus: a study of septo-hippocampal neurons in freely moving rats. s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

SUMMARY

Memory is not unique to the human mind. In contrast. The ability to store information is essential to all animal life. Without it, the most elementary forms of learning would have been impossible. Even the most 'primitive' animals like worms and molluscs possess this basic capacity. More complex animals, like man and other mammals, have the capacity to store matrices of associations between perceptions in time, space and context.

Our understanding of the memory of such patterns is limited. However, it is recognised that memory in mammals depends on a structure in the brain called the hippocampus, and on the molecule acetylcholine. Both serious damage to the hippocampus and loss of the nerve cells (neurons) that secrete acetylcholine in the brain result in diminished capacity to store new information.

The hippocampus, named after its resemblance to the sea horse (*hippo* = horse, *campus* = sea monster; Greek), is a structure imbedded in the temporal lobe of the cerebral cortex. It functions as a temporary store and a processor for new information. The acetylcholine secreting (cholinergic) fibres that reach the hippocampus play an important role in the storage of information in the hippocampus.

The cholinergic innervation of the hippocampus originates in the medial septum. This small structure lies in the lower-inner part of the forebrain and contains the cell-bodies that send their fibres to the hippocampus. The release of acetylcholine is determined largely by the electrical activity in septo-hippocampal fibres. In addition, acetylcholine released in the medial septum is also involved in the regulation of hippocampal activity.

Acetylcholine in the hippocampus has been the focus of a considerable research effort. Beside the scientific interest in the biological substrate of memory, these efforts are motivated by the discovery that degeneration of the hippocampus and its cholinergic innervation is an important feature of

Summary

Alzheimer's disease. Alzheimer's disease is the most common neurodegenerative disease, affecting about 20% of all individuals above 80 years of age. Disorientation and loss of short term memory, which are characteristic to the early stages of the disease, are largely attributed to low levels of acetylcholine in the hippocampus and cerebral cortex. Consequently, the pharmacological therapy of Alzheimer's disease consists mainly of drugs that elevate acetylcholine levels in the brain.

Despite the research efforts directed to the cholinergic innervation of the hippocampus, a primary aspect of this systems has remained relatively unexplored. There is little known about the factors that control the release of acetylcholine in living animals. The reason for this is that methods for measuring neurotransmitters in the functioning brain of experimental animals were not available until recently. This has changed with the development of intracerebral microdialysis in the beginning of the eighties. This method is essentially an application of semi-permeable membranes developed for the artificial kidney. A small-diameter membrane tube from an artificial kidney is sealed at one end and mounted on a hollow glass fibre. This construction, the dialysis probe, is implanted by surgery into the brain of an anaesthetised animal and fixed onto the skull. Artificial cerebrospinal (brain) fluid is pumped through the glass fibre and flows along the membrane to the outlet of the probe. Small molecules diffuse from the surrounding brain tissue into the probe through the membrane. The fluid is collected at the outlet and analysed in fractions. The compound to be measured is separated from other components and quantified.

The release of a neurotransmitter in the structure where the dialysis probe is placed can be estimated by measuring the amount of the neurotransmitter in subsequent dialysate fraction. In addition, dialysis probes can be used to administrate drugs directly into the brain. The drug is dissolved in the inflowing fluid and diffuses outwards into the surrounding brain tissue.

This thesis describes the development of a method for studying acetylcholine release from septo-hippocampal neurons in the rat. Two dialysis probes were implanted in each rat, one in the hippocampus and one in the medial septum. Both probes were used for measuring acetylcholine, as well as for the administration of drugs. All the experiments had dual objectives. On one hand, to identify the major factors that control the release of acetylcholine, and on the other hand, to evaluate and explore the possibilities of this experimental design. We anticipated that the design would provide unique in-

formation on the regulation of acetylcholine release in alive and unrestrained animals.

First, we had to prove that our experimental design worked properly. This was done in three stages. First we showed that acetylcholine measured in dialysis samples was released from nerve endings. Then, we demonstrated that acetylcholine levels in the hippocampus were related to the electrical activity in septo-hippocampal fibres. Finally, we showed that administration of drugs via the dialysis probe in the medial septum affected acetylcholine release in the hippocampus. The above results are presented in **Chapter 2**.

Neurons communicate by releasing neurotransmitters. A Neurotransmitter is released from nerve endings and binds to receptors on a receiving neuron. The binding of the neurotransmitter to the receptor alters the activity of the receiving neuron. Receptors are also used as targets for drugs that influence the communication between neurones.

The release of acetylcholine from septo-hippocampal neurons is modulated by the receptors present on these neurons. The next stage of the research was to identify these receptors by measuring acetylcholine during the administration of drugs that block or stimulate different receptors types.

Most neurons have receptors for the neurotransmitter that they release (autoreceptors). Neurotransmitters are believed to regulate their own release via these autoreceptors (autoinhibition). The effect of autoreceptors on the release of acetylcholine in the hippocampus and medial septum is the subject of **Chapter 3**. Autoinhibition was present in both structures. Moreover, autoinhibition was found to determine the release of acetylcholine to great extent. However, autoreceptors in the medial septum did not affect the electrical activity in septo-hippocampal fibres.

In **Chapters 4 and 5**, we investigated the involvement of two classes of receptors that were likely to affect acetylcholine release in the hippocampus, namely, receptors for the amino-acid neurotransmitters glutamate and GABA. GABA was found to inhibit acetylcholine release in the medial septum and the hippocampus continuously. The results also indicated that glutamate stimulates acetylcholine via receptors in the medial septum, but reduces acetylcholine release via receptors in the hippocampus. Glutamate receptors were, however, not activated by endogenous glutamate. It was unclear in which circumstances glutamate receptors could be involved in the regulation of acetylcholine release.

Summary

Benzodiazepines, (e.g. Valium), are commonly used as tranquillisers, sleep-enhancing drugs and antiepileptics, and are parts of most kinds of anaesthesia. Benzodiazepines act by enhancing the effect of GABA. One of their side effects is a temporary relapse of memory. The underlying cause might be inhibition of acetylcholine release in the hippocampus. To test this hypothesis, we administered a benzodiazepine by injection under the skin (subcutaneously), or via the dialysis probe in the medial septum. Subcutaneous, but not intraseptal administration, decreased acetylcholine release. This result support the idea that a reduction in hippocampal acetylcholine levels might contribute to memory impairment by benzodiazepines. However, GABA receptor in the medial septum do not appear to be involved in this effect. Perhaps the reduction in acetylcholine is caused by the effects of benzodiazepines on behaviour and the sensitivity to the environment, and is not directly related to the GABAergic inhibition of septo-hippocampal neurons.

Changes in acetylcholine release in the hippocampus correspond to changes in behavioural activity. Periods of activity are accompanied by increases in acetylcholine levels in the hippocampus and cerebral cortex. This relationship between acetylcholine and behaviour caused problems in earlier stages of the research, because some of the drugs that were administered also induced behavioural activity. This activity affects acetylcholine release and therefore, it is likely to interfere with the direct effect of the drug on cholinergic neurons. This problems has been resolved by using anaesthetised animals in some experiments.

In the last stage of the research, the same association between acetylcholine and behaviour was utilised to investigate the functional significance of receptors. Rats were activated by removing them from their home cage and holding them in the hand for several minutes. This procedure was also performed during the administration of drugs via the dialysis probe. The involvement of a receptor in behaviour-induced changes in acetylcholine release was investigated by comparing the behaviour-induced acetylcholine release in the presence of a drug to that in controls.

In **Chapter 7**, we investigated the involvement of GABA and glutamate in the induction of acetylcholine release during behaviour. In **Chapter 4**, we already showed that GABA represses acetylcholine release continuously via receptors in the medial septum. Reduction of this inhibition might stimulate cholinergic neurons during behavioural activity. This hypothesis was not supported by our results, but the involvement of GABA receptors could not

be excluded because the administration of a GABA analogue in the medial septum increased the activity of the rats.

Blocking of glutamate receptors in the medial septum reduced the increase in acetylcholine as induced by the behavioural procedure. Thus, the stimulation of glutamate receptors in the medial septum is one of the mechanisms that cause acetylcholine to increase during behavioural activity.

To measure acetylcholine with our method, it is necessary to elevate the levels of acetylcholine in the brain. This was attained by adding a compound that reduces the metabolic degradation of acetylcholine (an acetylcholinesterase inhibitor) to the fluid in the dialysis probe. The resulting increase in acetylcholine levels is likely to increase the level of autoinhibition, and might alter the normal pattern and pharmacological properties of acetylcholine release. Acetylcholinesterase inhibitors are also used in the therapy of Alzheimer's disease to increase acetylcholine levels in the brain. Autoinhibition might restrict the effects of acetylcholinesterase inhibitors on acetylcholine levels and repress functional changes in acetylcholine release. The relationship between acetylcholinesterase inhibition, autoinhibition and behaviour-induced acetylcholine release is the subject of **Chapter 6**.

First, the relation between acetylcholinesterase inhibition and acetylcholine levels was characterised by administration three different doses of an inhibitor through the dialysis probe. The involvement of autoinhibition was visualised by simultaneous administration of a drug that blocks autoreceptors. Rats were activated by the behavioural procedure in all these conditions in order to investigate the effects of acetylcholinesterase inhibition and autoinhibition on physiological changes in acetylcholine release.

Autoinhibition became activated only when acetylcholine levels were increased several fold of normal levels by acetylcholinesterase inhibition. However, when autoinhibition became active, it reduced the effect of acetylcholinesterase inhibition on acetylcholine levels substantially. Hence, large increases in acetylcholine levels are prevented by autoinhibition.

Behaviour-induced increase in acetylcholine levels became relatively smaller at higher levels of acetylcholinesterase inhibition. However, it was unclear whether this was the result of autoinhibition. In the presence of the lower doses of acetylcholinesterase inhibitors, autoinhibition was not detected even when the animals were activated and acetylcholine release increased. Only in presence of higher doses of the acetylcholinesterase inhibitor, behaviour-induced increase in acetylcholine release resulted in additional autoinhibition only in the. It is difficult to understand the normal

Summary

function of autoreceptors if they are not activated in physiological conditions. Perhaps their function is to prevent acetylcholine from 'overshooting' to toxic values.

The most important conclusion of this thesis is that the experimental design we developed provides unique information on the cholinergic neurons that innervate the hippocampus. Receptors that affect the release of acetylcholine release can be identified and studied in alive and unrestrained animals. Moreover, the method allows us to investigate the role of these receptors in normal changes in acetylcholine release. Perhaps, the experimental design can be applied in Alzheimer research. For example, new and current methods to elevate acetylcholine levels in the brain can be compared and evaluated.

SAM

Geb
forma
is elke
daaron
Meer
tussen

Onz
end da
een st
choline
zenuw
vermog

De
(hippo
(tempo
'proces
linerge
de opsl

Acet
septum
uitlope
pus wo
septo-h
het me
van ne

Er is
hippoca
ologisch
king o